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Metoclopramide inhibits trigeminovascular activation: evidence for effective acute attack treatment in migraine

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Background/aim: Metoclopramide is an effective and commonly used medication in acute migraine treatment but an experimental evidence base is lacking. We aimed to investigate the antimigraine effect of metoclopramide in a migraine model and whether the analgesic effect of metoclopramide was likely to be D_2 receptor-mediated.

Materials and methods: Cortical spreading depression (CSD) was used to model migraine in adult male Wistar rats. Five CSDs were induced by pinprick. Metoclopramide (two different doses), raclopride, or 0.9% saline were administered 30 min before CSD induction. Two hours after the experiments, brain tissues were examined for c-fos activation.

Results: In metoclopramide groups brain stem c-fos expression was significantly lower than in the CSD side of the saline group ($P = 0.002$). In the raclopride group, ipsilateral brain stem c-fos expression was also lower than in the saline group ($P = 0.002$). No difference in c-fos expression in the ipsilateral trigeminal nucleus caudalis between the raclopride and metoclopramide groups was observed ($P > 0.05$).

Conclusion: Metoclopramide is shown to suppress trigeminovascular activation for the first time, providing an experimental basis for its role in migraine. The analgesic effect of metoclopramide is likely to be mediated by D_2 receptors since raclopride, a selective D_2 receptor antagonist, suppresses trigeminovascular activation similarly.

Key words: Metoclopramide, trigeminal nucleus caudalis, D_2 receptors, raclopride, trigeminovascular system, migraine, rat

1. Introduction

The most common drugs prescribed for first-line therapy in acute migraine attacks are simple oral analgesics and metoclopramide in children and adults admitted to the emergency department (1–3). Metoclopramide, an antiemetic drug, is a D_2 receptor antagonist, an HT_4 receptor agonist, and a weak $5HT_3$ antagonist. The use of antiemetics in migraine attacks is recommended to treat nausea and to improve the resorption of analgesics. Metoclopramide also has an analgesic property in migraine with level B efficacy (4,5). In clinical practice, metoclopramide has higher efficacy intravenously (6). In a metaanalysis performed with phase IV studies, sumatriptan and ketorolac were not found superior to iv metoclopramide in complete pain relief and rescue drug needs (7). Though metoclopramide provides an effective and safe treatment that is widely used to abort migraine attacks (6), its mechanisms of action are still unclear since very limited experimental studies are available.

We aimed to investigate the antimigraine effect of metoclopramide in a migraine model in rats and whether the analgesic effect of metoclopramide was likely to be mediated through dopamine D_2 receptors. Cortical spreading depression (CSD), which is a well-known model for migraine, activates trigeminovascular afferents and induces early gene product c-fos expression in the ipsilateral cortex and brain stem trigeminal nucleus caudalis (TNC) laminae I and II during the process of lateralized headache. CSD-induced brain stem c-fos expression has been suppressed by efficient antimigraine drugs (8,9). We also examined the CSD-induced activation of the meningeal layers pia-arachnoid and dura mater as all those tissues are innervated by the trigeminal nerve.

2. Materials and methods

All animal procedures were performed after obtaining approval from the Animal Care Committee of the Gazi

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University Medical Center in accordance with the Turkish Animal Welfare Act. The study was conducted in the Gazi University Neuropsychiatry Center's Neuroscience Laboratory. Adult male Wistar rats (200–250 g) were used. Rats were kept in transparent cages exposed to 12 h of daylight and 12 h of darkness with a fixed temperature of 22 ± 2 °C.

The animals were administered 2 different doses of intraperitoneal metoclopramide (0.1 mg/kg or 1 mg/kg) and 0.5 mg/kg raclopride 30 min before CSD. In the vehicle group 0.9% saline was used. Brain tissues were prepared for c-fos immunohistochemical analysis. Dura mater, cortical sections with pia-arachnoid mater, and brain stem TNC sections were investigated following c-fos immunohistochemistry staining.

The c-fos in the lamina I and II brain stem of the CSD side and contralateral side was counted under light microscope and compared between the 0.9% saline, metoclopramide, and raclopride groups.

2.1. Animals and experimental setup

Each group consisted of seven adult male Wistar rats. Rats were fasted one night before and were anesthetized with intraperitoneal sodium thiopental (30 mg/kg). Rats were maintained at 37 °C by a heating blanket using a rectal probe. After being placed into a stereotaxic frame, a craniotomy at the frontal region 2–4 mm lateral and 1–3 mm anterior to the bregma was opened.

2.2. CSD protocol

A bipolar parallel electrode was placed 900 µm into the cortex and an electrocorticogram along with a DC recording was obtained during CSD. CSD was induced by pinprick method 1.5 mm anterior to the recording electrode.

Five CSDs were evoked at intervals of 15–20 min for c-fos expression and rats were perfused transcardially 2 h after the last CSD. Tissues were sectioned, immunohistochemistry was performed, and c-fos-positive neurons were counted as described below.

2.3. Drug administration

In the vehicle group 0.9% saline, in the metoclopramide groups metoclopramide at doses of 0.1 and 1 mg/kg, and in the raclopride (Sigma, USA) group 0.5 mg/kg raclopride was administered intraperitoneally 30 min before craniotomy.

2.4. Preparation of tissues

Two hours after CSD, rats were perfused transcardially with heparinized 0.9% NaCl followed by cold 0.1 M phosphate-buffered 4% paraformaldehyde solution. Brains with brain stem and dura mater were postfixed overnight (4 °C) in 4% paraformaldehyde solution and then separated from the cranium and incubated in 30% sucrose solution for 3–4 days. When brain tissues sank to the bottom of the solution, brain stems were frozen by dry ice, and every 150 µm sections (50 µm thick) were taken for immunostaining (Leica) from C_2 to the obex of the brain stem. Brain cortical sections were similarly obtained 50 µm thick at the piriform cortex level and immunohistochemistry followed.

2.5. Immunohistochemistry (c-fos)

Free floating sections of the brain stem, cortex, and dura mater from all groups were stained with positive and negative controls. Sections were washed in 0.1 M PBS solution 3 times for 10 min each. They were then placed in H_2O_2 to block endoperoxidase activity at room temperature for 30 min. This solution was prepared with 10 mL of PBS and 100 µL of 30% H_2O_2. After sections were washed in 0.1 M PBS solution 3 times for 10 min again, protein blockage was performed at room temperature for 2 h with 9800 µL of PBS, 200 µL of goat serum, and 30 µL of Triton X mixture. For the primary antibody phase, c-fos antibody (Calbiochem, USA, at 1/5000 dilution) was added to the blockage solution and incubated at 4 °C for one night. The next day, sections were washed in 0.1 M PBS solution 3 times for 10 min and incubated in secondary antibody, 1 mL of 2% goat serum, 1/600 biotinylated rabbit IgG, and 0.3% Triton X solution, at room temperature for 2 h. Then they were washed in 0.1 M PBS solution 3 times for 10 min. One drop of solution A and 1 drop of solution B was added to 5 mL of PBS and sections were incubated at room temperature for 2 h in this solution. Then they were washed in 0.1 M PBS solution again and stained with DAB for 1–5 min until a light brown color was observed. Free-floating sections or dura mater were then mounted to microscope slides and left for drying. After air-drying, they were put in 75% alcohol for 10 min, 90% alcohol for 10 min, and 100% alcohol for 10 min. They were dried in air for 10 min, then dipped into xylene for 10–20 min, then closed with a cover slip.

2.6. c-fos-positive cell count in TNC and pia-arachnoid mater

The c-fos-positive neurons were counted by a blinded observer under light microscope (Nikon ECLIPSE TE 2000-U). Counting was done by the help of a rat atlas at all levels of the trigeminal nucleus and nociceptive laminae I and II. Sections at obex level (obex ± 0.5 mm), below the obex brain stem (between obex – 0.5 mm and obex – 3 mm), and upper cervical levels (between obex – 3 mm and obex – 5 mm) were evaluated. Coronal brain sections were also investigated under light microscopy for c-fos-positive cells in the pia-arachnoid membrane.

2.7. Statistics

Statistical tests were performed with SPSS 15.0. Normalization of data was investigated by Kolmogorov–Smirnov test. c-fos expression data between groups were

analyzed by Kruskal–Wallis test when there were more than two independent groups and by Mann–Whitney U test when there were two independent groups. Brain stem c-fos expression in the control group between ipsilateral and contralateral sides was analyzed by Wilcoxon signed-rank test. Statistical significance was assumed at $P < 0.05$.

3. Results

In the 0.9% saline group, brain stem c-fos expression of the CSD side was significantly higher than that of the contralateral side (97.7 ± 8.8 vs. 50.0 ± 6.4, P = 0.028) (Figures 1A and 1B). In both metoclopramide groups, brain stem TNC sections' c-fos expression was significantly lower than that of the vehicle group's CSD side (metoclopramide 0.1 mg/kg 62.7 ± 4.4, 1 mg/kg 62.8 ± 4.3, P = 0.002) (Table). In the raclopride group, ipsilateral CSD induced c-fos expression in the TNC was also lower than that of the vehicle group (57.5 ± 5.2, P = 0.002). There was no significant difference in c-fos expression in ipsilateral TNC between raclopride and metoclopramide 0.1 mg/kg and 1 mg/kg groups (P = 0.132 and P = 0.093, respectively). No significant difference was found between contralateral c-fos expression between 0.9% saline, metoclopramide, and raclopride groups (P = 0.372).

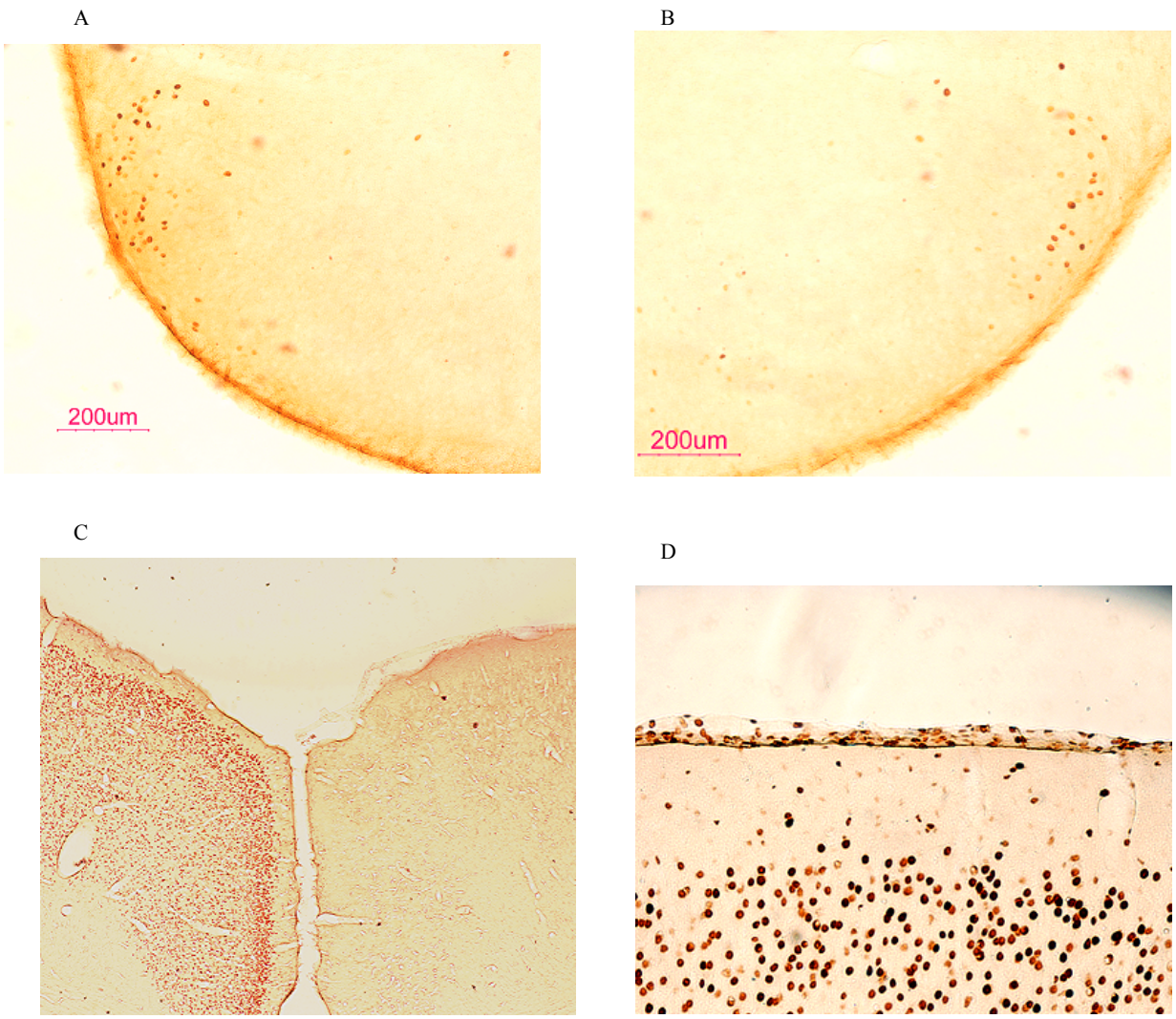

Figure 1. CSD-induced c-fos expression in the cerebral cortex and brain stem. A) c-fos expression of ipsilateral TNC sections of the control (0.9% saline) group. c-fos-positive neurons were particularly found in nociceptive lamina I and II at the ventral part of the ophthalmic division of the trigeminal nerve. B) c-fos expression of contralateral TNC sections of the control (0.9% saline) group. C) c-fos expression within ipsilateral cerebral cortex and pia-arachnoid mater, which is used to verify CSD (40×). No c-fos expression was detected in the contralateral cortex and pia-arachnoid membranes. D) Pia-arachnoid c-fos activation at a higher magnification (100×).

Table. c-fos expression in ipsilateral and contralateral TNC sections in 0.9% saline, metoclopramide, and raclopride groups.

	Control (n = 7)	Metoclopramide 0.1 mg/kg (n = 7)	Metoclopramide 1 mg/kg (n = 7)	Raclopride 0.5 mg/kg (n = 7)	P-value†
Ipsilateral TNC	97.7 ± 8.8[abc]	62.7 ± 4.4[a]	62.8 ± 4.3[b]	57.5 ± 5.2[c]	0.002
Contralateral TNC	50.0 ± 6.4	52.5 ± 3.4	50.7 ± 4.6	49.0 ± 2.8	0.372
TNC ratio, ipsilateral/contralateral	1.97 ± 0.24 [abc]	1.20 ± 0.11[a]	1.25 ± 0.20[b]	1.18 ± 0.10[c]	0.004

† Kruskal–Wallis test.
a: There is a statistically significant difference between the 0.1 mg/kg metoclopramide group and the control group (P = 0.002). b: There is a statistically significant difference between the 1 mg/kg metoclopramide group and the control group (P = 0.002). c. There is a statistically significant difference between the 0.5 mg/kg raclopride group and the control group (P = 0.002).

Our findings showed dense c-fos expression in the ipsilateral cortex and surrounding pia-arachnoid membrane (Figure 1C), but none in the outer meningeal membrane called the dura mater. CSD did not induce c-fos expression in the contralateral cortex and pia-arachnoid membrane (Figure 1D).

4. Discussion

Metoclopramide is one of the commonly used mediations in acute migraine treatment but an experimental evidence base is lacking. This study supplies an experimental basis for metoclopramide usage in migraine. The main finding is that CSD-induced c-fos expression in ipsilateral TNC is lowered significantly when metoclopramide is administered intraperitoneally. Raclopride also lowers the CSD-induced c-fos expression in the ipsilateral TNC. There was no difference between the metoclopramide and raclopride groups, giving rise to the idea that metoclopramide may show its analgesic effect in migraines through D_2 receptors.

Dopamine seems to be involved in migraine with regards to premonitory symptoms such as drowsiness, yawning, and food cravings; concomitant symptoms during migraine attack such as nausea and vomiting; and postdrome symptoms such as tiredness and mood changes (10). In addition, migraineurs show hypersensitivity to dopamine agonists, such as apomorphine, which produces more yawning (11), and piribedil, which causes nausea, vomiting, and sudden fall in blood pressure in migraine patients (12). Domperidone, a dopamine antagonist, can prevent migraine when it is taken in the premonitory phase (13). D_2 receptor antagonists such as metoclopramide, domperidone, chlorpromazine, and prochlorperazine are used to cease or reduce the severity of a migraine attack. All of these findings point toward the role of dopamine in migraine. We provided the evidence for the first time that two D_2 receptor antagonists, namely metoclopramide and raclopride, effectively blocked the CSD-induced trigeminal brain stem nuclei activation. Researchers in one experimental study, by using intravital microscopy, did not detect a dopaminergic effect on dural vessel vasodilation induced by direct electrical stimulation (14).

Dopamine receptors are present in the trigeminocervical complex and dopaminergic drugs contribute to the modulation of neuronal firing in this area; this is associated with migraine pathophysiology (10). Additionally, in migraines gastrokinetic dysfunction is present, so D_2 antagonists probably attenuate the headache severity in migraines directly and via improving gastric absorption, thus increasing the absorption of the analgesic drug (10).

Metoclopramide also is a $5HT_3$ receptor antagonist and, since $5HT_3$ receptors are located in the area postrema, these receptors have potential roles in nausea and emesis and $5HT_3$ receptor antagonism increases the antiemetic effect of metoclopramide (15). It was also suggested that the migraine headache relief mechanism of metoclopramide is due to $5HT_3$ receptor antagonism (16). In our study, rats receiving the selective D_2 receptor raclopride had a similar decrease in CSD-induced c-fos expression in the ipsilateral TNC, suggesting that the analgesic effect of metoclopramide is probably mediated by D_2 receptors and the other receptors that metoclopramide acts on do not seem to have a major impact on headache.

Metoclopramide, a widely used agent in migraine attacks, is shown here to suppress trigeminovascular activation in the brain stem for the first time. Efficiency of metoclopramide has been shown experimentally by less c-fos expression in metoclopramide-treated CSD groups. The underlying mechanism of the clinical effect of metoclopramide has been revealed for the first time, which will bring more understanding to migraine pathophysiology.

CSD leads to the activation of pia-arachnoid cells (inner meningeal layers) although dural (outer meningeal layer)

cells remain unaffected, and substantial c-fos expression is detected in the pia-arachnoid membrane but not in the dura mater.

The limitation of the study is that, even if the antimigraine effect of metoclopramide is shown to be likely mediated by D_2 receptors, we do not know if $5HT_3$ and $5HT_4$ receptors contribute to this antimigraine effect since no medications acting on these receptors were evaluated in this study.

In conclusion, metoclopramide and raclopride did not prevent the occurrence of CSD, as shown with dense c-fos immunohistochemistry, but they suppressed trigeminovascular system activation as demonstrated by reduced c-fos expression in the TNC. Metoclopramide is shown to suppress trigeminovascular activation in the brain stem for the first time. This study supports the clinical use of metoclopramide in migraine patients by providing experimental evidence in acute attack treatment. The headache-attenuating effect of metoclopramide seems to be likely mediated by D_2 receptor antagonism since raclopride, a selective D_2 receptor antagonist, suppresses trigeminovascular activation, similar to metoclopramide.

References

1. Colman I, Brown MD, Innes GD, Grafstein E, Roberts TE, Rowe BH. Parenteral metoclopramide for acute migraine: meta-analysis of randomised controlled trials. BMJ 2004; 329: 1369-1373.
2. Richer L, Graham L, Klassen T, Rowe B. Emergency department management of acute migraine in children in Canada: a practice variation study. Headache 2007; 47: 703-710.
3. Schulman EA, Dermott KF. Sumatriptan plus metoclopramide in triptan-nonresponsive migraineurs. Headache 2003; 43: 729-733.
4. Bolay H, Durham P. Biological sciences for headache; pharmacology. In: Nappi G, Moskowitz M, editors. Headache: Handbook of Clinical Neurology Series. Vol. 97. Amsterdam, the Netherlands: Elsevier; 2010. pp. 47-71.
5. Evers S, Afra J, Frese A, Goadsby PJ, Linde M, May A, Sándor PS; European Federation of Neurological Societies. EFNS guideline on the drug treatment of migraine-revised report of an EFNS task force. Eur J Neurol 2009; 16: 968-981.
6. Friedman BW, Esses D, Solorzano C, Dua N, Greenwald P, Radulescu R, Chang E, Hochberg M, Campbell C, Aghera A et al. A randomized controlled trial of prochlorperazine versus metoclopramide for treatment of acute migraine. Ann Emerg Med 2008; 52: 399-406.
7. Eken C. Critical reappraisal of intravenous metoclopramide in migraine attack: a systematic review and meta-analysis. Am J Emerg Med 2015; 33: 331-337.
8. Bolay H, Reuter U, Dunn AK, Huang ZH, Boas DA, Moskowitz MA. Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. Nat Med 2002; 8: 136-142.
9. Tepe N, Filiz A, Dilekoz E, Akcali D, Sara Y, Charles A, Bolay H. The thalamic reticular nucleus is activated by cortical spreading depression in freely moving rats: prevention by acute valproate administration. Eur J Neurosci 2015; 41: 120-128.
10. Akerman S, Goadsby PJ. Dopamine and migraine: biology and clinical implications. Cephalalgia 2007; 27: 1308-1314.
11. Blin O, Azulay JP, Masson G, Aubrespy G, Serratrice G. Apomorphine-induced yawning in migraine patients: enhanced responsiveness. Clin Neuropharmacol 1991; 14; 91-95.
12. Bès A, Dupui P, Güell A, Bessoles G, Géraud G. Pharmacological exploration of dopamine hypersensitivity in migraine patients. Int J Clin Pharmacol Res 1986; 6: 189-192.
13. Amery WK, Waelkens J. Prevention of the last chance: an alternative pharmacologic treatment of migraine. Headache 1983; 23: 37-38.
14. Akerman S, Goadsby PJ. The role of dopamine in a model of trigeminovascular nociception. J Pharmacol Exp Ther. 2005; 314: 162-169.
15. Walkembach J, Brüss M, Urban BW, Barann M. Interactions of metoclopramide and ergotamine with human $5\text{-}HT_{3A}$ receptors and human 5-HT reuptake carriers. Br J Pharmacol 2005; 146: 543-552.
16. Schwarzberg MN. Application of metoclopramide specificity in migraine attacks therapy. Headache 1994; 34: 439-441.